

Shared and Specific Susceptibility Loci for Schizophrenia and Bipolar Disorder

A Dense Genome Scan in Eastern Quebec Families

Maziade et al., Molecular Psychiatry (2005)

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Part I: Background & Study Pedigrees

Study Objective

Primary Goal: Identify shared and disorder-specific susceptibility loci for schizophrenia (SZ) and bipolar disorder (BP)

Key Questions:

- Do SZ and BP **share** common genetic susceptibility alleles?
- Are there alleles **specific** to each disorder?

Why study SZ and BP together?

- Family studies show co-aggregation of SZ and BP within pedigrees
- Previous linkage studies analyzed the disorders separately
- Liddle: look at symptom dimensions rather than disease categories
- Separate analysis may cause diagnostic misclassification to break segregation patterns within pedigrees

Background: The Diseases

Bipolar I Disorder (BP):

- Mood disorder with manic and depressive episodes
- Lifetime prevalence of 0.6%; Bipolar spectrum disorders: 1-2%
- **Highly heritable (60-85%)**
- ~70% reported psychotic symptoms

Schizophrenia (SZ):

- Key features include hallucinations, delusions, disorganized speech, abnormal motor behavior, diminished emotional expression
- Lifetime prevalence in population is 0.3-0.7%
- **High heritability (70-80%)**
- **Schizoaffective Disorder:**
 - Schizophrenia with mood symptoms (mania and/or depression)
 - lifetime prevalence is ~0.3%

Diagnostic blindness: ensures that clinical diagnosis is assigned independently of family structure

Complex Disorders

- Non-Mendelian inheritance with incomplete penetrance
 - Carrying a susceptibility allele does not guarantee disease expression
- Genetic heterogeneity
 - Similar clinical phenotypes may result from different susceptibility allele or mutations across families
- Phenocopies
 - Similar clinical phenotype induced by environmental factors
 - Eg., prenatal or perinatal adversities including stress, infection, or other medical complications
- Other risk factors: neurodevelopmental factors, substance exposure, psychosocial stressors, etc.

Study Population: Eastern Quebec Families

Sample Characteristics:

- 21 multigenerational families from Eastern Quebec, Canada
- Average of 6 affected individuals (SZ or BP) per family
- N = 480 family members (DNA available)
- 47 deceased affected ancestors included in pedigree analysis

Family Selection Criteria:

- At least one first-degree relative with same disorder as proband
- At least two additional affected relatives (first/second/third-degree)
- At least four affected members per family

Family Composition:

- 7 SZ pedigrees (85%+ affected by SZ spectrum among ill members)
- 6 BP pedigrees (85%+ affected by BP spectrum among ill members)
- 8 mixed pedigrees (both SZ and BP)

Part II: Methods Overview

Methods: The Big Picture

Overall Analytical Strategy:

Step 1: Define phenotypes

Step 2: Specify transmission models

Step 3: Perform linkage analysis with Mod Score approach

Step 4: Apply multiple testing correction

Step 5: Conduct heterogeneity analysis

Background Concept: What is Linkage?

Linkage = The tendency for genes/markers close together on a chromosome to be inherited together

- Linkage analysis requires pedigree data because it detects co-segregation of chromosomal regions through meioses

Key Principle (from Lecture 4):

- If a marker is **physically close** to a disease gene, they will **co-segregate** within families
- The closer they are, the less likely recombination occurs between them

Recombination Fraction (θ):

- θ = probability of recombination between two loci
- $\theta = 0.5$: loci are unlinked (different chromosomes or far apart)
- $\theta < 0.5$: loci are linked
- $\theta = 0$: loci are completely linked (no recombination)

Background Concept: Microsatellite Markers

What are microsatellites?

- Known positions
- Scattered throughout the genome

Why use microsatellites for linkage analysis?

- High heterozygosity → more **informative meioses**
- Short tandem repeats (STRs) of 2-4 nucleotides (e.g., CA-CA-CA-CA, or CA-CA, etc.)
- A pedigree contributes to LOD score only if key individuals are heterozygous
- More alleles = better ability to track inheritance

This study used:

- 607 microsatellite markers
- 350 markers at 10cM resolution (initial scan)
- 257 additional markers in promising regions

Methods Step 1: Phenotype Definitions

Three diagnostic classes analyzed:

Phenotype	Narrow Definition	Broad Definition
SZ	SZ only (N=71)	SZ+schizophreniform+schizotypal (N=81)
BP	BP I only (N=48)	BP I+BP II+recurrent depression (N=72)
CL	SZ + BP I + schizoaffective (N=134)	CL narrow+depression +schizotypal (N=169)

CL = “Common Locus” phenotype

- Combines SZ and BP phenotypes
- Tests hypothesis that some loci are shared by both disorders

Methods Step 2: Transmission Models

Model-based linkage analysis requires specifying:

- ① **Mode of inheritance:** Dominant vs. Recessive
- ② **Disease allele frequency:** How common is the risk allele?
- ③ **Penetrance:** $P(\text{affected} \mid \text{genotype})$

Two models used in this study:

Parameter	Dominant Model	Recessive Model
Disease allele frequency	0.01	0.10
Penetrance (high age)	0.70	0.70
Phenocopy rate	0.20	0.10

Why test multiple models?

- True inheritance pattern is unknown for complex diseases
- Maximizing over models increases power but also Type I error

Background Concept: LOD Score

LOD (Log of Odds) Score measures evidence for linkage:

$$LOD(\theta) = \log_{10} \frac{L(\theta)}{L(\theta = 0.5)} = \log_{10} \frac{\text{Likelihood under linkage}}{\text{Likelihood under no linkage}}$$

When penetrance $f < 1$ or phenocopy rate $f_0 > 0$, the likelihood:

$$L(\theta) = \sum_{\text{genotypes}} P(\text{phenotypes} | \text{genotypes}, f, f_0) \cdot P(\text{genotypes} | \theta)$$

where for each individual i : $P(\text{phenotypes} | \text{genotypes}, f, f_0) = \prod_i P(Y_i | G_i)$

$$P(Y_i | G_i) = \begin{cases} f^{Y_i}(1-f)^{1-Y_i} & \text{if } G_i = \text{carrier} \\ f_0^{Y_i}(1-f_0)^{1-Y_i} & \text{if } G_i = \text{non-carrier} \end{cases}$$

Methods Step 3: Linkage Analysis Details

Two-point vs. Multipoint Analysis:

- **Two-point:** Tests linkage between disease and ONE marker
- **Three-point (multipoint):** Uses flanking markers together
 - More informative, extracts more information
 - Each marker included in two three-point analyses

Mod Score Approach:

- Maximize LOD score over θ AND over model parameters
- $$\text{Mod Score} = \max_{\theta, \text{models}} LOD(\theta)$$
- Tests 8 combinations: 2 phenotype levels (narrow vs broad) \times 2 transmission models (dominant vs recessive) \times 2 analysis types (affect/unaffected vs affected-only)

Software: FASTLINK (version of LINKAGE)

Methods Step 4: Multiple Testing Correction

The Problem:

- 607 markers \times 3 phenotypes \times 8 model combinations = many tests!
- High risk of false positives

Solution: Lander & Kruglyak (1995) Thresholds

Category	Z-score	Interpretation
Significant	≥ 4.0	Genomewide significant ($p < 0.05$)
Suggestive	≥ 2.6	Expected once per genome scan by chance
Confirmatory	≥ 1.9	Only in regions with prior evidence

Additional correction in this study:

- Raised thresholds by 0.70 (following Hodge et al.)
- Because: 24 non-independent analyses per marker

Methods Step 5: Heterogeneity Analysis

What is genetic heterogeneity?

- Same phenotype caused by different genes in different families
- Common in complex diseases

Problem: If only some families are linked to a locus, the overall LOD score may be diluted

Solution: Admixture Model (HOMOG program)

- Estimates proportion of families linked (α)
- Calculates HLOD (heterogeneity LOD)
- Applied to signals with mod score > 1.9

$$HLOD = \log_{10} [\alpha \cdot L(\theta) + (1 - \alpha) \cdot L(\theta = 0.5)] - \log_{10} L(\theta = 0.5)$$

Part III: Results & Figures

Genetic Nomenclature: Loci and Markers

- **Chromosomal Location** (e.g., 15q11.1)
 - **15**: the chromosome number
 - **q**: the “long arm” of the chromosome (p stands for the “short arm”)
 - **11.1**: the specific band and sub-band on that arm, numbered in increasing order as the distance from the centromere (the center) increases
- **Genetic Markers** (e.g., D15S122)
 - **D**: DNA
 - **15**: located on Chromosome 15
 - **S**: a single-copy sequence (a unique genomic locus)
 - **122**: the unique ID number for this specific marker
- **The Relationship:**
 - **15q11.1** defines a broad chromosomal region
 - **D15S122** is a precise genetic marker within that region, used to test linkage to a nearby disease locus

Results (see Table 1)

- **Significant Linkage Findings** (MOD Score ≥ 4.0):
 - **BP**: 15q11.1 (D15S122), 18q12.3 (D18S1145), 16p12.3 (D16S410)
 - **CL**: 15q26 (D15S1014), 18q21.1 (D18S472)
- **Suggestive Linkage Findings** (MOD Score ≥ 2.6):
 - **BP**: 12q23.1 (D12S1030), 3q21.2 (D3S3023), 10p13 (D10S674)
 - **SZ**: 6p22.3 (D6S334), 18q21.1 (D18S851), 13q13.3 (D13S1491)
 - **CL**: 16p13.1 (D16S3041), 2q22.1 (D2S298), 13q14.1 (D13S1247)
- **Novelty and Replication** (see Table 3):
 - **Novel Discovery**: the linkage signal at **15q26** for the shared phenotype
 - **Confirmatory Evidence** (MOD score ≥ 1.9): the finding at **3q21.2** for BP, **6p22.3** for SZ, **10p15-p11** for BP and SZ, **18q12.3-q32** for BP and SZ, etc.

Table 2: Family Contributions to the Genetic Signal

- The linkage signal is supported by several pedigrees rather than driven by a single influential family

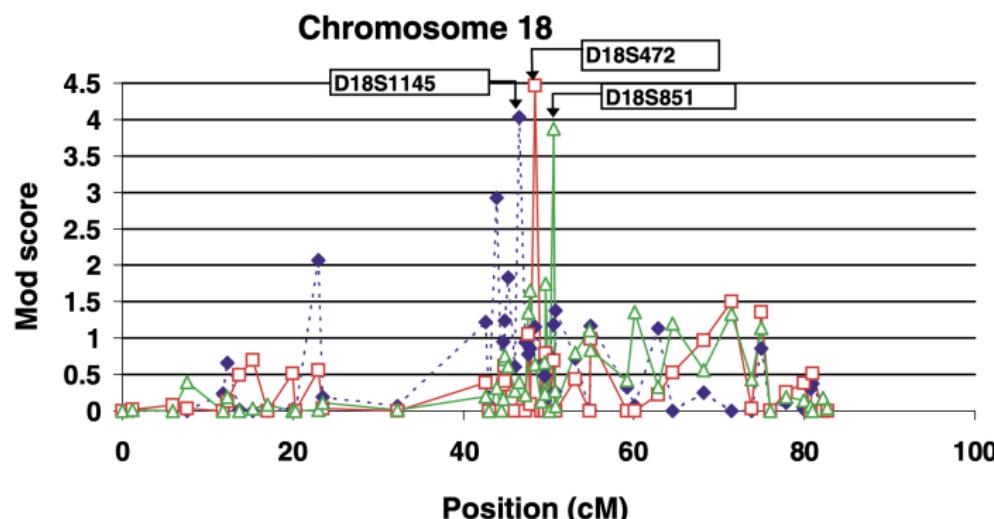
Table 2 Percentage of families according to their individual lod score contributing to the significant and suggestive total mod scores

Marker	Mod score	Phenotype	Individual pedigree lod score interval					Highest family lod score
			lod<0.1	0.1≤lod<0.4	0.4≤lod<0.8	0.8≤lod<1.2	1.2≤lod	
<i>Genomewide significant linkage</i>								
D15S122	4.59	BP broad	29%	21%	36%	14%	0%	1.04
D15S1014	4.55	CL narrow	29%	48%	19%	0%	5%	1.22
D16S410	4.05	BP narrow	21%	21%	57%	0%	0%	0.61
D16S3041	3.66	CL broad	33%	29%	38%	0%	0%	0.67
D18S1145	4.10	BP broad	43%	21%	21%	7%	7%	1.21
D18S472	4.46	CL narrow	33%	43%	24%	0%	0%	0.71
D18S851	3.87	SZ narrow	33%	53%	7%	0%	7%	2.03
<i>Suggestive linkage</i>								
D2S298	2.76	CL broad	52%	38%	5%	0%	5%	1.41
D3S3023	2.68	BP narrow	57%	14%	21%	7%	0%	1.04
D6S334	2.82	SZ narrow	40%	33%	7%	20%	0%	1.06
D10S674	2.66	BP narrow	50%	7%	43%	0%	0%	0.79
D12S1030	3.53	BP broad	21%	57%	14%	7%	0%	0.81
D13S1491	2.96	SZ narrow	33%	40%	13%	13%	0%	1.00
D13S1247	2.74	CL narrow	57%	19%	14%	5%	5%	1.69

For the analyses of the BP, the SZ and the CL phenotypes, the numbers of pedigrees under analysis were respectively 14, 15 and 21.

Figure 1: Visualizing Linkage across Chromosomes

- **X-axis:** physical distance along the chromosome (measured in cM)
- **Y-axis:** MOD score (the strength of the evidence)
- **Example:** Chromosome 18 shows overlapping linkage peaks for SZ, BP and the combined phenotype, suggesting a shared susceptibility locus rather than diagnosis-specific genes



Part IV: Discussion

Evidence for Shared Loci Between SZ and BP

- Data indicates overlapping susceptibility regions, suggesting that SZ and BP may share a common biological etiology in specific areas
- **Key Shared Hotspots:**
 - **Chromosome 18q:** showed consistent significant or suggestive linkage across SZ, BP, and combined phenotypes
 - **Chromosome 15q:** suggested the presence of one or more susceptibility genes contributing to either or both disorders
 - **Chromosome 16p:** primarily driven by BP and the shared phenotype, indicating a potential shared susceptibility locus; absence of a signal for SZ likely reflects diagnostic misclassification within pedigrees
- These results strongly support the hypothesis that some genetic risk factors are **not specific to one diagnosis** but are shared across the spectrum of these two major psychoses

Limitations

1. Statistical Thresholds

- Unclear if these criteria fit a small number of large families
- The effect of a two-step scan (sparse then dense) is hard to simulate

2. Sensitivity of Heterogeneity

- Current tests may fail to detect between-family heterogeneity due to small number of families

3. Complexity of Replication

- Difficult to define “true replication” across studies due to variations in:
 - Sampling & Diagnostics
 - Statistical methods & Genetic markers

Implications & Future Direction

- **Combination Model:** SZ and BP specificity arises from unique combinations of multiple shared genes
- **Modifier Gene Model:** SZ and BP share core genes, and specific modifier genes determine the final clinical phenotype
 - **Necessary but Not Sufficient:** Genes at loci like **6p22 (SZ)** or **16p12 (BP)** require interactions with other genes to trigger the disorder
 - **Affected-Only vs Affect/Unaffected:** More significant signals in AO analysis compared to AU
- **Future Directions**
 - The presence of multiple signals in one sample may allow for the study of gene-gene interactions (epistases)
 - Expand statistical power using a second sample of 500 members, ultimately identifying defective genes and paving the way for new treatments

Questions for Class Discussion

- ① Why did the authors use **model-based** (parametric) linkage analysis instead of model-free methods for this study? What are the trade-offs?
- ② The authors used both **narrow and broad** phenotype definitions. How might the phenotype definitions affect LOD scores and power?
- ③ We learned that LOD scores require specifying **penetrance** values. This study used age-dependent penetrance. Why is this important for late-onset disorders?
- ④ The maximum family LOD score was only ~2.0 (Table 2), yet the combined scores reached significance. What does this tell us about **genetic heterogeneity** of these disorders?
- ⑤ This is a **two-stage design** (initial sparse scan + dense follow-up). What are the advantages of this approach compared to performing dense genotyping from the start?